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(54) Title: AUTOLOGOUS TREATMENT OF DEGENERATED DISC WITH CELLS

(57) Abstract: The present invention relates to administering autologous uncultured cells into a diseased intervertebral disc.

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AUTOLOGOUS TREATMENT OF DEGENERATED DISC WITH CELLS

RELATED APPLICATIONS

This application is a continuation of U.S. Application No. 10/714,594, filed November 14, 2003, which is a continuation-in-part application of U.S. Application No. 10/714,559, filed November 13, 2003. The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The natural intervertebral disc contains a jelly-like nucleus pulposus surrounded by a fibrous annulus fibrosus. Under an axial load, the nucleus pulposus compresses and radially transfers that load to the annulus fibrosus. The laminated nature of the annulus fibrosus provides it with a high tensile strength and so allows it to expand radially in response to this transferred load.

In a healthy intervertebral disc, the cells within the nucleus pulposus form only about one percent of the disc tissue by volume. These cells produce an extracellular matrix (ECM) containing a high percentage of proteoglycans. These proteoglycans contain sulfated functional groups that retain water, thereby providing the nucleus pulposus with its cushioning qualities. The nucleus pulposus cells may also secrete small amounts of cytokines as well as matrix metalloproteinases (MMPs). These cytokines and MMPs help regulate the metabolism of the nucleus pulposus cells.

In some instances of disc degeneration disease (DDD), gradual degeneration of the intervertebral disc is caused by mechanical instabilities in other portions of the spine. In these instances, increased loads and pressures on the nucleus pulposus cause the cells within the disc (or invading macrophages) to emit larger than normal amounts of the above-mentioned cytokines. In other instances of DDD, genetic factors or apoptosis can also cause a decline in the number of disc cells and/or release of toxic amounts of these cytokines and MMPs. In some instances, the pumping action of the disc may malfunction (due to, for example, a decrease in the proteoglycan concentration within the nucleus pulposus), thereby retarding the flow

of nutrients into the disc as well as the flow of waste products out of the disc. This reduced capacity to provide nutrients to the cells and eliminate waste may result in decreased cell viability and metabolism resulting in further degradation of the ECM along with the accumulation of high levels of toxins that may cause nerve irritation and pain.

As DDD progresses, toxic levels of the cytokines and MMPs present in the nucleus pulposus begin to degrade the ECM. In particular, the MMPs (as mediated by the cytokines) begin cleaving the water-retaining portions of the proteoglycans, thereby reducing its water-retaining capabilities. This degradation leads to a less flexible nucleus pulposus, and so changes the loading pattern within the disc, thereby possibly causing delamination of the annulus fibrosus. These changes cause more mechanical instability, thereby causing the cells to emit even more cytokines, typically thereby upregulating MMPs. As this destructive cascade continues and DDD further progresses, the disc begins to bulge ("a herniated disc"), and then ultimately ruptures, causing the nucleus pulposus to contact the spinal cord and produce pain.

U.S. Patent No. 6,352,557 ("Ferree") teaches adding therapeutic substances such as nucleus pulposus cells to morselized extra-cellular matrix obtained from donors, and injecting that combination into an intervertebral disc. However, the cells first need to be cultured and then added to the donor matrix prior to implantation into the diseased disc. This process requires a delay in the patient's treatment in addition to subjecting the patient to two separate procedures. The first procedure is to harvest the cells, which then require culturing. Following the culturing the cells are implanted into the patient.

U.S. Patent No. 6,340,369 ("Ferree II") teaches harvesting live intervertebral disc cells from a patient, culturing the cells and transplanting them into the affected disc. Ferree II further teaches that the cells can be combined with Type II collagen-glycosaminoglycan matrix or Type I collagen-glycosaminoglycan matrix depending on whether the cells are harvested from the nucleus pulposus (NP) or annulus fibrosus (AF). Also Ferree II suggests adding one or more therapeutic substances to cells prior to transplantation. As an alternate source for cells, Ferree proposes using precursor cells of NP or AF cells, chondrocytes or other living cells that function

like or could differentiate into NP or AF cells. Throughout, Ferree teaches that the harvested cells are cultured prior to transplantation.

Alini, *Eur. Spine J.*, 11(Supp.2): S215-220 (2002), suggests that injection of a biomatrix embedded with cells will have the potential to restore functionality to the disc. Alini's experiments are directed to isolating cells from the nucleus pulposus and culturing them. Alini also suggests other sources of cells including disc cells from allogenic donors and autologous stem cells. His teachings suggest that stem cells would be an ideal source but that there are no known methods for culturing the stem cells such that they would differentiate into nucleus pulposus cells prior to implantation. In essence, Alini requires that cells be cultured prior to implantation.

Russell (Abstract 27 ISSLS 2003) reports conducting an experiment to determine whether mesenchymal stem cells (MSCs) could be directed to present disc chondrocyte phenotypes. Russell found that adult human MSCs were induced to differentiate along a chondrocytic phenotype when mediated by culture conditions and also by addition of TGF-B1.

Sakai (Abstract 24 ISSLS 2003) reports evaluating whether autologous transplantation of MSCs to the disc would prevent disc degeneration. Using rabbits, MSCs were isolated from the bone marrow and cultured for 2 weeks prior to transplantation. Results showed significant disc preservation.

Sakai, *Biomaterials*, 24: 3531-3541 (2003) describes using a final cell density of 1 x 10^6 cells/ml, to inject 0.04 ml of solution in which autogenous cultured MSCs were embedded through a 27-gauge insulin injector to each disc. Proliferation of cells after transplantation was found to be successful.

Sobajima (Abstract 43 ISSLS 2002) studied the feasibility of stem cell therapy for DDD. Human NP cells were isolated from patients undergoing disc surgery and were co-cultured with either MSCs from patients undergoing hip surgery or muscle derived stem cells from mice. The data demonstrated a synergistic effect between stem cells and nucleus pulposus cells, resulting in upregulated proteoglycan synthesis *in vivo*.

Ganey, *Eur Spine J*, 11(Suppl.2):S206-S214 (2002), reported on surgeries conducted in Germany where cells were harvested from portions of a patient's disc

after discectomy. The cells were then cultured and returned for transplantation into the patient at a later date.

Sander *et al.* in US Patent Application Publication 2003/0069639, teaches using tissue biopsies taken from a patient as a source to harvest cells for implantation into a degenerated disc.

All of the teachings cited above require culturing of cells prior to implantation, which, in turn, necessitates a delay in treating the patient's degenerating disc.

SUMMARY OF THE INVENTION

The present inventors have developed an intra-operative procedure for efficaciously treating degenerative disc disease by introducing autologous uncultured cells, (e.g., mesenchymal stem cells or chondrocytes or fibroblasts) into the patient's disc. This procedure provides immediate point of care treatment for the patient.

In accordance with one embodiment of the present invention, the present inventors have developed a method of treating an intervertebral disc in which cells harvested from the patient's bone marrow are then introduced into the degenerated disc to differentiate into nucleus pulposus and/or annulus fibrosus cells present in the disc, thereby increasing the number of those cells present in the disc. In some embodiments, the implantation of the cells into the disc can occur immediately following the harvesting of the cells, so that the patient can avoid undergoing a first procedure to harvest the cells, waiting for the cells to be cultured (which may take several weeks), and then returning for a second procedure to implant the cultured cells into the disc.

There are believed to be several advantages to introducing cells to a targeted disc. A primary function of the cells is to produce extra-cellular matrix. As described above, there are several factors that result in cell death or malfunction, which in turn contribute to the degradation of this matrix. One strategy to rebuild or regenerate the extra-cellular matrix is to increase the number of viable functioning disc cells producing the matrix. The inventors believe that the plasticity phenomenon of the mesenchymal stem cells (MSCs) makes them an ideal choice of cell type for differentiating into disc cells after implantation into the targeted disc.

The cells may become nucleus pulposus (NP) and/or annulus fibrosus (AF) cells that will be capable of producing the necessary extra-cellular matrix within the disc. In addition, at the time of implantation, the cells may be combined with other therapeutic agents such as growth factors to help the cells survive, once inside the disc.

Accordingly, in one aspect of the present invention, there is provided a method of treating degenerative disc disease in an intervertebral disc, comprising harvesting MSCs from a patient, and introducing the viable MSCs, without having to culture them, into the same patient's degenerated intervertebral disc, where the cells will proliferate and differentiate into nucleus pulposus and/or annulus fibrosus cells.

In some embodiments, the cells are delivered alone or via a carrier. In other embodiments, the cells are delivered along with an additional therapeutic agent or substance such as a growth factor to the disc.

DETAILED DESCRIPTION OF THE INVENTION

Because DDD is a continuous process, the degenerating disc to which the cells are administered may be in any one of a number of degenerative states. Accordingly, the degenerating disc may be an intact disc. The degenerating disc may be a herniated disc (*i.e.,* wherein a portion of the annulus fibrosus has a bulge). The degenerating disc may be a ruptured disc (*i.e.,* wherein the annulus fibrosus has ruptured and the bulk nucleus pulposus has exuded). The degenerating disc may be delaminated *(i.e.,* wherein adjacent layers of the annulus fibrosus have separated). The degenerating disc may have fissures (*i.e.*, wherein the annulus fibrosus has fine cracks or tears through which selected molecules from the nucleus pulposus can leak). In all of these degenerative states, the extra-cellular matrix of either the AF or NP is also degrading.

The present invention is directed to intra-operatively providing healthy, viable autologous mesenchymal stem cells (MSCs) to a degenerated intervertebral disc of a patient. The cells may be delivered to either the nucleus pulposus or the annulus fibrosus or both for repair and restoration of each respective extra-cellular matrix.

The inventors believe that MSCs provide a special advantage for administration into a degenerating disc because they possess properties that will help them to more readily survive the relatively harsh environment present in the degenerating disc. Specifically, MSCs have a desirable level of plasticity that gives them the ability to proliferate and differentiate into NP and AF cells.

In one embodiment, the MSCs are obtained from the patient's own bone marrow. In other embodiments, adipose or muscle tissue may be the source of MSCs. In some embodiments, the MSCs to be administered to the disc are provided in a concentrated form. When provided in concentrated form, the cells can be uncultured. Uncultured, concentrated MSCs can be readily obtained by centrifugation, filtration (selective retention), or immunoabsorption. When filtration is selected, the methods disclosed in U.S. Patent No. 6,049,026 ("Muschler"), the contents of which are incorporated by reference in their entirety, can be used. For example, a bone marrow aspirate suspension can be passed through a porous, biocompatible, implantable substrate to provide a composite bone graft having an enriched population of tissue progenitor cells. In some embodiments, the matrix used to filter and concentrate the MSCs is also co-administered into the nucleus pulposus or annulus fibrosus as a therapeutic agent. If this matrix has suitable mechanical properties, it can be used to restore the height of the disc space that was lost during the degradation process. The cells may be injected at the same time or concurrently with the matrix in the targeted area of the disc.

The volume of aspirated bone marrow obtained to harvest the MSCs is preferably between about 5 cc to about 100 cc. This volume is then used during the concentration process to concentrate the MSCs.

When centrifugation is selected, the methods disclosed by Connolly *et al.* can be used. Incorporated by reference in its entirety is *Development of an Osteogenic Bone Marrow Preparation*, JBJS 71-A (No.5) (June 1989). In this rabbit study, Connolly reported that centrifugation of 7-10 ml of bone marrow yielded an average of 3.6×10^6 nucleated cells per milliliter in final cell suspension.

When the cells are concentrated using the centrifugation process, they are deliverable to the disc in a pellet form in suspension. In another embodiment, the cells are delivered using a carrier. The carrier can comprise, or can be selected

from, the group consisting of beads, microspheres, nanospheres, hydrogels, gels, polymers, ceramics, collagen and platelet gels.

The carrier, in solid or fluid form, can carry the cells in several different ways. The cells can be embedded, encapsulated, suspended or attached to the surface of the carrier. In one embodiment, the carrier encapsulates the cells, provides nutrients, and protects the cells when they are delivered inside the disc. After a period of time inside the disc, the carrier degrades and releases the cells. Specific types of the various carriers are described below.

In some embodiments, the mesenchymal stem cells are provided in a sustained release device (*i.e.,* sustained delivery device). The administered formulation can comprise the sustained release device. The sustained release device is adapted to remain within the disc for a prolonged period and slowly release the mesenchymal stem cells contained therein to the surrounding environment. This mode of delivery allows the mesenchymal stem cells to remain in therapeutically effective amounts within the disc for a prolonged period. One or more additional therapeutic agents can also be delivered by a sustained delivery device.

Synthetic scaffolds, such as fumaric-acid based scaffolds, have been designed and tailored to allow for attraction of certain cells and to provide direction for the cells to differentiate in desired areas. The cells can also be embedded in the scaffold and then injected into the target area without affecting the viability or proliferation of the cells. After implantation of the fumaric-acid based scaffold, it degrades over time and no further surgery is necessary to remove the scaffold.

Carriers can also comprise hydrogels. The cells are encapsulated in the polymer chains of the hydrogel after gelation. Hydrogels can be delivered in a minimally invasive manner, such as injection to the target area. The hydrogel is also resorbed by the body. Hydrogel properties such as degradation time, cell adhesion behavior and spatial accumulation of extracellular matrix can be altered through chemical and processing modifications.

Hydrogels suitable for use in the present invention include water-containing gels, *i.e.,* polymers characterized by hydrophilicity and insolubility in water. See, for instance, "Hydrogels", pages 458-459, in *Concise Encyclopedia of Polymer Science and Engineering,* Eds. Mark *et al.,* Wiley and Sons (1990), the disclosure of

which is incorporated herein by reference in its entirety. Although their use is optional in the present invention, the inclusion of hydrogels can be highly advantageous since they tend to possess a number of desirable qualities. By virtue of their hydrophilic, water-containing nature, hydrogels can house viable cells, such as mesenchymal stem cells, and can assist with load bearing capabilities of the disc.

In one embodiment, the hydrogel is a fine, powdery synthetic hydrogel. Suitable hydrogels exhibit an optimal combination of properties such as compatibility with the matrix polymer of choice, and biocompatability. The hydrogel can include any one or more of the following: polysaccharides, proteins, polyphosphazenes, poly(oxyethylene)-poly(oxypropylene) block polymers, poly(oxyethylene)-poly(oxypropylene) block polymers of ethylene diamine, poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers.

In general, these polymers are at least partially soluble in aqueous solutions, *e.g.*, water, or aqueous alcohol solutions that have charged side groups, or a monovalent ionic salt thereof. There are many examples of polymers with acidic side groups that can be reacted with cations, *e.g.*, poly(phosphazenes), poly(acrylic acids), and poly(methacrylic acids). Examples of acidic groups include carboxylic acid groups, sulfonic acid groups, and halogenated (preferably fluorinated) alcohol groups. Examples of polymers with basic side groups that can react with anions are poly(vinyl amines), poly(vinyl pyridine), and poly(vinyl imidazole).

In accordance with the present invention, there is provided a method of treating degenerative disc disease in an intervertebral disc having a nucleus pulposus, comprising administering autologous uncultured mesenchymal stem cells into a degenerated intervertebral disc.

In one embodiment, the autologous mesenchymal stem cells are harvested before they are administered into the disc.

In accordance with one aspect of the invention, the mesenchymal stem cells can be delivered into the disc space with at least one (an) additional therapeutic agent, such as an agent to aid in the proliferation and differentiation of the cells. There can be, for example, one additional therapeutic agent (*i.e.*, a second therapeutic agent) or there can be multiple additional therapeutic agents (*e.g.*, second

and third therapeutic agents). The additional therapeutic agent may be delivered simultaneously with the mesenchymal stem cells. In another embodiment, the additional therapeutic agent is delivered after administering the mesenchymal stem cells to the disc. In yet another, the additional therapeutic agent is administered first, *i.e.*, prior to administering the mesenchymal stem cells to the disc.

The same carrier may also be used to deliver the cells and the additional therapeutic agent. In some embodiments, the cells are located on the surface of the carrier and the additional therapeutic agent is placed inside the carrier. In other embodiments, the cells and the additional therapeutic agent may be delivered using different carriers.

Other additional therapeutic agents which may be added to the disc include, but are not limited to: vitamins and other nutritional supplements; hormones; glycoproteins; fibronectin; peptides and proteins; carbohydrates (simple and/or complex); proteoglycans; oligonucleotides (sense and/or antisense DNA and/or RNA); bone morphogenetic proteins (BMPs); differentiation factors; antibodies (for example, antibodies to infectious agents, tumors, drugs or hormones); gene therapy reagents; and anti-cancer agents. Genetically altered cells and/or other cells may also be included in the matrix of this invention. If desired, substances such as pain killers (*i.e.*, analgesics) and narcotics may also be admixed with the carrier for delivery and release to the disc space.

In some embodiments, growth factors are additional therapeutic agents. As used herein, the term "growth factor" encompasses any cellular product that modulates the growth or differentiation of other cells, particularly connective tissue progenitor cells. The growth factors that may be used in accordance with the present invention include, but are not limited to, members of the fibroblast growth factor family, including acidic and basic fibroblast growth factor (FGF-1 and FGF-2) and FGF-4, members of the platelet-derived growth factor (PDGF) family, including PDGF-AB, PDGF-BB and PDGF-AA; EGFs, members of the insulin-like growth factor (IGF) family, including IGF-I and -II; the TGF-β superfamily, including TGF-β1, 2 and 3 (including MP-52), osteoid-inducing factor (OIF), angiogenin(s), endothelins, hepatocyte growth factor and keratinocyte growth factor; members of the bone morphogenetic proteins (BMPs) BMP-1, BMP-3, BMP-2, OP-1, BMP-2A,

BMP-2B, BMP-4, BMP-7 and BMP-14; HBGF-1 and HBGF-2; growth differentiation factors (GDFs), members of the hedgehog family of proteins, including indian, sonic and desert hedgehog; ADMP-1; GDF-5; and members of the colony-stimulating factor (CSF) family, including CSF-1, G-CSF, and GM-CSF; and isoforms thereof. The growth factor can be autologous such as those included in platelet rich plasma or obtained commercially. In one embodiment, the growth factor is administered in an amount effective to repair disc tissue.

In some embodiments, the growth factor is selected from the group consisting of TGF-β, bFGF, and IGF-1. These growth factors are believed to promote regeneration of the nucleus pulposus, or stimulate proliferation and/or differentiation of chondrocytes, as well as extracellular matrix secretion. In one embodiment, the growth factor is TGF-β. More preferably, TGF-β is administered in an amount of between about 10 ng/ml and about 5000 ng/ml, for example, between about 50 ng/ml and about 500 ng/ml, *e.g.*, between about 100 ng/ml and about 300 ng/ml.

In one embodiment, at least one of the additional therapeutic agents is TGF-β1. In one embodiment, another additional therapeutic agent is FGF.

In some embodiments, platelet concentrate is provided as an additional therapeutic agent. In one embodiment, the growth factors released by the platelets are present in an amount at least two-fold (*e.g.*, four-fold) greater than the amount found in the blood from which the platelets were taken. In some embodiments, the platelet concentrate is autologous. In some embodiments, the platelet concentrate is platelet rich plasma (PRP). PRP is advantageous because it contains growth factors that can restimulate the growth of the ECM, and because its fibrin matrix provides a suitable scaffold for new tissue growth.

Therefore, in accordance with the present invention, there is provided a method of treating degenerative disc disease in an intervertebral disc having a nucleus pulposus, comprising:

a) administering autologous uncultured mesenchymal stem cells into the degenerating disc; and

b) transdiscally administering at least one additional therapeutic agent into the degenerating disc.

For the purposes of the present invention, "transdiscal administration" includes, but is not limited to:

a) injecting a formulation into the nucleus pulposus of a degenerating disc, such as a relatively intact degenerating disc;

b) injecting a formulation into the annulus fibrosus of a degenerating disc, such as a relatively intact degenerating disc;

c) providing a formulation in a patch attached to an outer wall of the annulus fibrosus,

d) providing a formulation in a depot at a location outside but closely adjacent to an outer wall of the annulus fibrosus ("trans-annular administration"); and

e) providing the formulation in a depot at a location outside but closely adjacent to an endplate of an adjacent vertebral body ("trans-endplate administration").

Also in accordance with the present invention, there is provided a formulation for treating degenerative disc disease, comprising:

a) autologous uncultured mesenchymal stem cells; and

b) at least one additional therapeutic agent,

wherein the formulation is present in an amount suitable for administration into a degenerating disc.

Also in accordance with the present invention, there is provided a device for delivering a formulation for treating degenerative disc disease to the disc comprising:

a) a chamber containing the formulation comprising autologous uncultured mesenchymal stem cells and at least one additional therapeutic agent; and

b) a delivery port in fluid communication with the chamber and adapted to administer the formulation to the disc.

In some embodiments, the cells may be introduced (*i.e.*, administered) into the nucleus pulposus or the annulus fibrosus depending on which extra-cellular matrix needs rebuilding. In other embodiments, the cells may be introduced into

both regions of the disc. Specific therapeutic agents may be selected depending on the region of the disc where the cells are going to be delivered.

In some embodiments, the cells alone are administered (*e.g.*, injected) into the disc through a needle, such as a small bore needle. Alternatively, the formulation can also be injected into the disc using the same small bore needle. In some embodiments, the needle has a bore of about 22 gauge or less, so that the possibilities of producing a herniation are mitigated. For example, the needle can have a bore of about 24 gauge or less, so that the possibilities of producing a herniation are even further mitigated.

If the volume of the direct injection of the cells or formulation is sufficiently high so as to cause a concern of overpressurizing the nucleus pulposus, then it is preferred that at least a portion of the nucleus pulposus be removed prior to administration (*i.e.*, direct injection) of the mesenchymal stem cells. In some embodiments, the volume of removed nucleus pulposus is substantially similar to the volume of the formulation to be injected. For example, the volume of removed nucleus pulposus can be within about 80-120% of the volume of the formulation to be injected. In addition, this procedure has the added benefit of at least partially removing some degenerated disc from the patient.

When injecting the mesenchymal stem cells into the nucleus pulposus, it is desirable that the volume of drug (*i.e.*, formulation of cells suspended in growth medium or a carrier) delivered be between about 0.5 ml and about 3.0 ml comprising cells suspended in growth medium or a carrier. When injected in these smaller quantities, it is believed that the added or replaced volume will not cause an appreciable pressure increase in the nucleus pulposus. Factors to consider when determining the volume of drug to be delivered include the size of the disc, the amount of disc removed and the concentration of the mesenchymal stem cells in the growth medium or carrier.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A method of treating degenerative disc disease in an intervertebral disc having a nucleus pulposus, comprising administering autologous uncultured cells into a degenerated intervertebral disc.

2. The method of Claim 1, wherein the cells are concentrated prior to being administered into the intervertebral disc.

3. The method of Claim 2, wherein the cells are concentrated by centrifugation.

4. The method of Claim 2, wherein the cells are concentrated by filtration.

5. The method of Claim 1, wherein the cells are administered to the disc using a carrier.

6. The method of Claim 5, wherein the carrier is selected from the group consisting of beads, microspheres, nanospheres, hydrogels, gels, polymers, ceramics, collagen and platelet gels.

7. The method of Claim 1, wherein an additional therapeutic agent is administered into the intervertebral disc.

8. The method of Claim 7, wherein the additional therapeutic agent is selected from the group consisting of growth factors, differentiation factors, and nutritional supplements.

9. The method of Claim 8, wherein the additional therapeutic agent is a growth factor.

10. The method of Claim 7, wherein the additional therapeutic agent and the cells are administered into the intervertebral disc using a carrier.

11. The method of Claim 10, wherein the carrier is selected from the group consisting of beads, microspheres, nanospheres, hydrogels, gels, polymers, ceramics, collagen and platelet gels.

12. The method of Claim 7, wherein the additional therapeutic agent is administered simultaneously with administering the cells to the disc.

13. The method of Claim 7, wherein the additional therapeutic agent is administered prior to administering the cells to the disc.

14. The method of Claim 7, wherein the additional therapeutic agent is administered after administering the cells to the disc.

15. The method of Claim 1, wherein the cells are administered into the intervertebral disc in a formulation with a volume of between about 0.5 ml and about 10 ml.

16. The method of Claim 10, wherein the carrier comprises a hydrogel.

17. The method of Claim 10, wherein the carrier comprises microspheres.

18. The method of Claim 1, wherein the additional therapeutic agent is TGF-β.

19. The method of Claim 1, wherein the therapeutic agent is platelet concentrate.

20. The method of Claim 1, wherein the cells are administered into the nucleus pulposus of the disc.

21. The method of Claim 1, wherein the cells are administered into the annulus fibrosus of the disc.

22. The method of Claim 1, wherein a portion of the nucleus pulposus is removed prior to administering the cells into the intervertebral disc.

23. The method of Claim 1, wherein the cells are administered through a needle.

24. The method of Claim 23, wherein the needle has a maximum gauge of about 24 gauge.

25. The method of claim 1 wherein the cells are selected from the group consisting of mesenchymal stem cells, chondrocytes and fibroblasts.

26. The method of claim 1 wherein the cells comprise mesenchymal stem cells.

27. A formulation for treating degenerative disc disease, comprising:

a) autologous uncultured mesenchymal stem cells; and

b) an additional therapeutic agent,

wherein the formulation is present in an amount suitable for administration into a degenerating disc.

28. The formulation of Claim 27, wherein the mesenchymal stem cells are provided in a concentrated form.

29. The formulation of Claim 27, wherein the additional therapeutic agent is a growth factor.

30. A device for administering the formulation of Claim 27 to a degenerated intervertebral disc comprising:

a) a chamber containing the formulation; and

b) a delivery port adapted to administer the formulation to the disc.

31. The method of Claim 1, wherein the formulation is administered in an amount of less than about 1 ml.

INTERNATIONAL SEARCH REPORT

Internat[redacted] Application No
PCT/US2004/037500

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/28 A61K35/32 A61K35/36 A61P19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAKAI D ET AL: "Transplantation of mesenchymal stem cells embedded in Atelocollagen<(>R) gel to the intervertebral disc: a potential therapeutic model for disc degeneration" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 24, no. 20, September 2003 (2003-09), pages 3531-3541, XP004429647 ISSN: 0142-9612 cited in the application abstract page 3532 page 3533, right-hand column, paragraph 3 page 3539, right-hand column ----- -/--	1-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2005	24/03/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 049 026 A (MUSCHLER ET AL) 11 April 2000 (2000-04-11) cited in the application column 2, line 28 - line 56 column 3, line 22 - line 32 column 4, line 18 - line 26 example 9	1-31
A	US 2003/069639 A1 (SANDER TOM ET AL) 10 April 2003 (2003-04-10) cited in the application abstract paragraph '0039! - paragraph '0040! last sentence , paragraph 44 paragraph '0052!; example 1	1-31
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P,X	WO 2004/022078 A (THE ROYAL VETERINARY COLLEGE; SMITH, ROGER, KENNETH, WHEALANDS; MCGARR) 18 March 2004 (2004-03-18) abstract page 5, line 16 - line 25 page 7, line 2 - line 5 page 8, line 4 - page 9, line 11 page 11, line 10 page 11, line 23 - line 26 page 12, line 3 - line 7 page 13, line 8 - line 19 claims 1,10-14,19,20	1-31
P,X	EP 1 464 307 A (DEPUY SPINE, INC) 6 October 2004 (2004-10-06) paragraph '0004! - paragraph '0007!	1-6,25, 26,30
L	US 2005/038001 A1 (ATTAWIA MOHAMED ET AL) 17 February 2005 (2005-02-17) Puts doubt on the validity of the claimed priorities paragraph '0081! - paragraph '0088!; claims 25-27,62-66	
L	US 2004/229786 A1 (ATTAWIA MOHAMED ET AL) 18 November 2004 (2004-11-18) Puts doubt on the validity of the claimed priorities	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/037500

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-26 and 31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/037500

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